The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed P. Mahoney

Dated 20 June 2003

Patents Form 1/77

Patents Act 1977
(Rule 16)

THE PATENT OFFICE
25 JUL 2002
RECEIVED

The **Patent Office**

1/77

26JUL02 E736212-6 D00524
P01/7700 0.00-0217305.2

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office
Cardiff Road
Newport
Gwent NP10 8QQ

1.	Your reference	G-32577P2/BCK 9926
2.	Patent application number *(The Patent Office will fill in this part)*	25 JUL 2002 0217305.2
3.	Full name, address and postcode of the or of each applicant *(underline all surnames)*	BIOCHEMIE GESELLSCHAFT MBH A-6250 KUNDL TIROL AUSTRIA
	Patent ADP number *(if you know it)*	
	If the applicant is a corporate body, give the country/state of its incorporation	AUSTRIA 08355158001
4.	Title of invention	Organic compounds
5.	Name of your agent *(If you have one)*	
	"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	B.A. YORKE & CO. CHARTERED PATENT AGENTS COOMB HOUSE, 7 ST. JOHN'S ROAD ISLEWORTH MIDDLESEX TW7 6NH
	Patents ADP number *(if you know it)*	1800001

6.	If you are declaring priority from one ore more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number	Country	Priority application number *(if you know it)*	Date of filing (day/month/year)
7.	If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application	Number of earlier application		Date of filing (day/month/year)

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

   a) any applicant named in part 3 is not an inventor, or

   b) there is an inventor who is not named as an applicant, or

   c) any named applicant is a corporate body.

   (see note (d))

   Yes

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	24
Claim(s)	3
Abstract	1
Drawing(s)	

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	ONE
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application

Signature B.A. Yorke & Co. Date

B.A. Yorke & Co. **25 July 2002**

12. Name and daytime telephone number of person to contact in the United Kingdom

Mrs. E. Cheetham
020 8560 5847

Warning

After an application for a patent has been filed. the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the united Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) *If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.*

b) *Write your answers in capital letters using black ink or you may type them.*

c) *If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.*

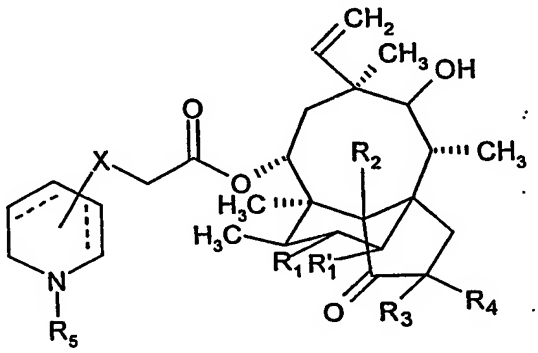
d) *Once you have filled in the form you must remember to sign and date it.*

e) *For details of the fee and ways to pay please contact the Patent Office.*

Organic Compounds

The present invention relates to organic compounds having pharmaceutical, e.g. antimicrobial activity; such as mutilins.

In one aspect the present invention provides a compound of formula



I

wherein

R_1 and R_1' are hydrogen or deuterium,

R_2, R_3 and R_4 are hydrogen or deuterium,

R_5 is hydrogen or a residue of an amino acid,

X is S or N-ALK,

one of the dotted lines is a bond and the other is no bond; or one of the dotted lines is a group -OAc attached to the piperidine ring in position 2, 3, 4, 5 or 6, and the other dotted line is no bond,

ALK is (C_{1-4})alkyl, e.g. methyl, and

Ac is hydrogen or (C_{2-12})acyl, e.g. a group -CO-CH_3.

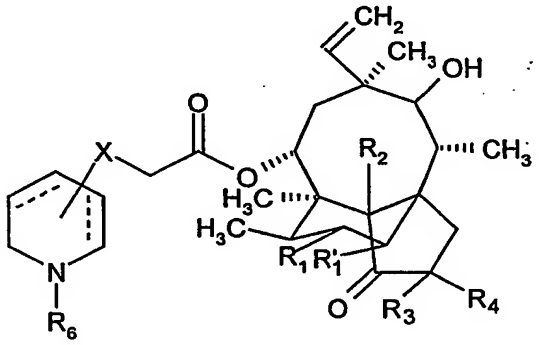
If a dotted line herein defined has the meaning of "no bond" said dotted line has no meaning, i.e. said dotted line is (regarded to be) not present.

In another aspect the present invention provides a compound of formula I selected from the group consisting of

- 14-O-[4-hydroxy-piperidin-3-yl-sulfanylacetylmutilin,
- 14-O-[3-hydroxy-piperidin-4-yl-sulfanylacetylmutilin,
- 14-O-[4-hydroxy-N-valyl-piperidin-3-yl]-sulfanylacetylmutilin, such as 14-O-[4-hydroxy-N-(R)-valyl-piperidin-3-yl]-sulfanylacetylmutilin, e.g. in the form of a hydrochloride,

- 14-O-[3-hydroxy-N-valyl-piperidin-4-yl]-sulfanylacetylmutilin, such as 14-O-[3-hydroxy-N-(R)-valyl-piperidin-4-yl]-sulfanylacetylmutilin, e.g. in the form of a hydrochloride,
- 14-O-[3-hydroxy-N-histidinyl-piperidin-4-yl]-sulfanylacetylmutilin, such as 14-O-[3-hydroxy-N-(R)-histidinyl-piperidin-4-yl]-sulfanylacetylmutilin, e.g. in the form of a dihydrochloride,
- 14-O-[3-hydroxy-N-valyl-piperidin-4-yl]-methylaminoacetylmutilin, such as 14-O-[3-hydroxy-N-(R)-valyl-piperidin-4-yl]-methylaminoacetylmutilin, e.g. in the form of a dihydrochloride,
- 14-O-[4-hydroxy-N-valyl-piperidin-3-yl]-methylaminoacetylmutilin, such as 14-O-[4-hydroxy-N-(R)-valyl-piperidin-3-yl]-methylaminoacetylmutilin, e.g. in the form of a dihydrochloride,
- 14-O-[N-valyl)-1,2,3,6-tetrahydropyridin-3-yl]-sulfanylacetylmutilin, such as 14-O-[N-(R)-valyl-1,2,3,6-tetrahydropyridin-3-yl]-sulfanylacetylmutilin, and
- 14-O-[N-valyl)-1,4,5,6-tetrahydropyridin-4-yl]-sulfanylacetylmutilin, such as 14-O-[N-(R)-valyl-1,4,5,6-tetrahydropyridin-4-yl]-sulfanylacetylmutilin.

In another aspect the present invention provides a compound of formula



II

wherein

R_1 and R_1' are hydrogen or deuterium,

R_2, R_3 and R_4 are hydrogen or deuterium,

R_6 is a protective group, or the residue of a protected amino acid,

X is S or N-ALK,

one of the dotted lines is a bond and the other is no bond; or one of the dotted lines is a group -OAc attached to the piperidine ring in position 2, 3, 4, 5 or 6, and the other dotted line is no bond,

ALK is (C_{1-4})alkyl, e.g. methyl, and

Ac is (C_{2-12})acyl, e.g. a group -CO-CH_3.

Protective group include protecting groups which may be, e.g. selectively, removed, if desired, and include protecting groups which are conventional in chemistry, e.g.

(pleuro)mutilin chemistry, preferably tert.butoxycarbonyl (BOC), e.g. which BOC can be removed e.g. by treatment with etheric HCl.

In another aspect the present invention provides a compound of formula II selected from the group consisting of

- 14-O-[N-BOC-4-hydroxy-piperidin-3-yl]-sulfanylacetylmutilin,
- 14-O-[N-BOC-3-hydroxy-piperidin-4-yl]-sulfanylacetylmutilin,
- 14-O-[4-hydroxy-N-BOC-piperidin-3-yl]-methylaminoacetylmutilin,
- 14-O-[3-hydroxy-N-BOC-piperidin-4-yl]-methylaminoacetylmutilin,
- 14-O-[N-BOC-1,4,5,6-tetrahydropyridin-4-yl]-sulfanylacetylmutilin, such as 14-O-[N-BOC-1,4,5,6-tetrahydropyridin-4(R*)-yl]-sulfanylacetylmutilin and 14-O-[N-BOC-1,4,5,6-tetrahydropyridin-4(S*)-yl]-sulfanylacetylmutilin,
- 14-O-[4-hydroxy-N-(N-BOC-valyl)-piperidin-3-yl]-sulfanylacetylmutilin, such as 14-O-[4-hydroxy-N-(N-BOC-(R)-valyl)-piperidin-3-yl]-sulfanylacetylmutilin, e.g. in the form of a hydrochloride,
- 14-O-[3-hydroxy-N-(N-BOC-valyl)-piperidin-4-yl]-sulfanylacetylmutilin, such as 14-O-[3-hydroxy-N-(N-BOC-(R)-valyl)-piperidin-4-yl]-sulfanylacetylmutilin, e.g. in the form of a hydrochloride,
- 14-O-[4-acetoxy-N-(N-BOC-valyl)-piperidin-3-yl]-sulfanylacetylmutilin, such as 14-O-[4-acetoxy-N-(N-BOC-(R)-valyl)-piperidin-3-yl]-sulfanylacetylmutilin, e.g. in the form of a hydrochloride,
- 14-O-[3-acetoxy-N-(N-BOC-valyl)-piperidin-4-yl]-sulfanylacetylmutilin, such as 14-O-[3-acetoxy-N-(N-BOC-(R)-valyl)-piperidin-4-yl]-sulfanylacetylmutilin, e.g. in the form of a hydrochloride,
- 14-O-[3-hydroxy-N-(N-BOC-histidinyl)-piperidin-4-yl]-sulfanylacetylmutilin, such as 14-O-[3-hydroxy-N-(N-BOC-(R)-histidinyl-piperidin-4-yl]-sulfanylacetylmutilin, e.g. in the form of a dihydrochloride.
- 14-O-[3-hydroxy-N-(N-BOC)-valyl-piperidin-4-yl]-methylaminoacetylmutilin, such as 14-O-[3-hydroxy-N-(N-BOC)-(R)-valyl-piperidin-4-yl]-methylaminoacetylmutilin, e.g. in the form of a dihydrochloride,
- 14-O-[4-hydroxy-N-(N-BOC)-valyl-piperidin-3-yl]-methylaminoacetylmutilin, such as 14-O-[4-hydroxy-N-(N-BOC)-(R)-valyl-piperidin-3-yl]-methylaminoacetylmutilin, e.g. in the form of a dihydrochloride,

- 14-O-[N-(N-BOC-valyl)-1,4,5,6-tetrahydropyridin-4-yl]-sulfanylacetylmutilin, such as 14-O-[N-(N-BOC-(R)-valyl)-1,4,5,6-tetrahydropyridin-4-yl]-sulfanylacetylmutilin,
- 14-O-[N-(N-BOC-valyl)-1,2,3,6-tetrahydropyridin-3-yl]-sulfanylacetylmutilin, such as 14-O-[N-(N-BOC-(R)-valyl)-1,2,3,6-tetrahydropyridin-3-yl]-sulfanylacetylmutilin.

In a compound of formula I or of formula II a carbon atom of the piperidine ring is bound to a group X. That group X may be in any position in the piperidine ring, e.g. in position 2, 3, 4, 5 or 6, preferably in position 3 or 4, if one of the dotted lines is a group -OAc; and if one of the dotted lines is a bond, the group X is attached to a $-CH_2-$ group in the piperidine ring. If one of the dotted line is a group -OAc in a compound of formula I, the -OAc group bound to the piperidine ring may be in any position, e.g. in position 2, 3, 4, 5 or 6, preferably in position 3 or 4. In a preferred group of compounds of formula I or of formula II one of the dotted line is a group -OAc and the group X is in position 3 and the group -OAc is in position 4; or the group X is in position 4 and the group -OAc is in position 3. In another preferred group of compounds of formula I or of formula II, one of the dotted lines is a bond and the group X is bound to a $-CH_2-$ group in the piperidine ring, preferably in position 5, if the bond is bridging positions 4 and 5; or in position 4, if the bond is bridging positions 2 and 3.

"A residue of an amino acid" as used herein means that in a compound of formula I the carbonyl group of said amino acid is bound to the N of the piperidine and the –OH group is missing, i.e. the N of the piperidine ring is acylated by the carboxylic group of an amino acid. Preferably the residue of an amino acid is valyl or histidinyl.

Compounds provided by the present invention, e.g. a compound of formula I or of formula II, are hereinafter designated as "compound(s) of (or compound(s) according to) the present invention". A compound of the present invention includes a compound in any form, e.g. in free form, in the form of a salt, in the form of a solvate and in the form of a salt and a solvate.

In another aspect the present invention provides a compound of formula I or of formula II in the form of a salt, or in the form of a salt and in the form of a solvate, or in the form of a solvate.

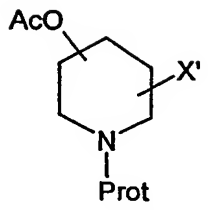
A salt of a compound of the present invention includes a pharmaceutically acceptable salt, e.g. including a metal salt or an acid addition salt. Metal salts include for example alkali or earth alkali salts; acid addition salts include salts of a compound of formula I with an acid, e.g. hydrogen fumaric acid, fumaric acid, naphthalin-1,5-sulphonic acid, hydrochloric acid, deuterochloric acid; e.g. hydrochloric acid or deuterochloric acid, preferably hydrochloric acid. A compound of the present invention may be converted into a corresponding compound in the form of a salt; and vice versa. A compound of the present invention in free form or in the form of a salt and in the form of a solvate may be converted into a corresponding compound in free form or in the form of a salt in unsolvated form; and vice versa.

A compound of of the present invention may exist in the form of isomers and mixtures thereof; e.g. optical isomers, diastereoisomers, cis trans conformers. A compound of the present invention may e.g. contain asymmetric carbon atoms and may thus exist in the form of diastereoisomeres and mixtures thereof, e.g. racemates. For example the group bound via the sulphur atom to the piperidine ring of a compound of formula I may be in the (R)- or in the (S)-configuration or in the form of mixtures thereof. E.g. the amine group of the amino acid residue, e.g. valyl or histidinyl residue, which is acylating the nitrogen atom of the piperidene ring may be in the (S)-configuration, in the (R)-configuration or in the form of mixtures therof. Isomeric mixtures may be separated as appropriate, e.g. according to a method as conventional, to obtain pure isomers. The present invention includes a compound of the present invention in any isomeric form and in any isomeric mixture.
Preferably the cofiguration in the mutilin ring of a compound of the present invention is the same as in a naturally produced mutilin.

In another aspect the present invention provides a process for the production of a compound of formula I or of formula II comprising the steps

A) for the production of a compound of formula I or of formula II wherein one of the dotted lines is a group -OAc, the other dotted line is no bond and the other residues are as defined above comprising the steps

a) reacting a compound of formula



III

wherein Prot is a protective group e.g. BOC and X' is -SH or -NH-Alk, with 22-O-tosyl-pleuromutilin and tert.But-OK to obtain a compound of formula II, wherein R_6 is a protective group, e.g. BOC,

b) deprotecting the nitrogen group of the piperidinyl ring in a compound obtained in step a), e.g. by use of etheric HCl, to obtain a compound of formula I, wherein R_5 is hydrogen, optionally

c) reacting a compound obtained in step b) with an amino-protected, e.g. BOC-protected, amino acid, e.g. valine or histidine, to obtain a compound of formula II, wherein R_6 is the residue of a protected amino acid, e.g. protected valine or histidine, preferably BOC-protected valine or histidine; optionally

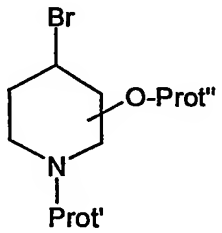
d) deprotecting the amino group of the amino acid residue of a compound obtained in step c) to obtain a compound of formula I, wherein R_5 is a residue of an amino acid, e.g. valyl or histidinyl; e.g. in the form of a salt, such as a hydrochloride; and optionally

e) introducing deuterium into a compound of formula I obtained in step d) to obtain a compound of formula I, wherein R_2, R_3 and R_4 are deuterium, and R_1, R'_1 and R_5 are as defined above.

B) for the production of a compound of formula I or of formula II wherein one of the dotted lines is a bond and the other dotted line is no bond,

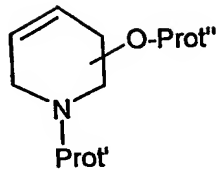
B1) if the dotted line is a bond bridging positions 4 and 5 in the piperidine ring,

a) reacting a compound of formula



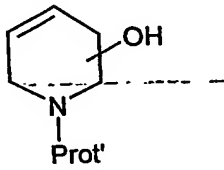
VIII

wherein Prot' is either a protecting group or the residue of a protected amino acid, e.g. wherein the residue of an protected amino acid is as defined above, and Prot" is a protecting group, e.g. $-OC-CH_3$, in the presence of DBU to obtain a compound of formula



V

b) removing the protecting group Prot'' from a compound of formula V to obtain a compound of formula



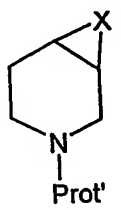
VI

c) reacting the hydroxy group in a compound iof formula VI with mesylchloride and the mesylate obtained with thiapleuromutiline or HN-alkyl-pleuromutilin to obtain a compound of formula II, wherein the dotted line bridging positions 4 and 5 in the piperidine ring is a bond and the other dotted line is no bond, Prot' is a protecting group or a the residue of a protected amino acid and the other residues are as defined above, and

d) removing the protecting Prot' if Prot' is a protecting group to obtain a compound of formula I wherein the dotted line bridging positions 4 and 5 in the piperidine ring is a bond and the other dotted line is no bond, R_5 is hydrogen and the other residues are as defined above; or removing the protecting group from the residue of the protected amino acid if Prot' is the residue of a protected amino acid, to obtain a compound of formula I wherein the dotted line bridging positions 4 and 5 in the piperidine ring is a bond and the other dotted line is no bond, R_5 is the residue of an amino acid and the other residues are as defined above;

B2) if the dotted line is a bond bridging positions 2 and 3 in the piperidine ring,

a) reacting a compound of formula

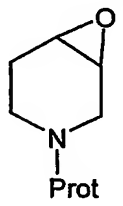


VII

wherein X and Prot' are as defined above, with 22-O-tosylpleuromutilin in the presence of n-butyl-lithium to obtain a compound of formula II,wherein the dotted line bridging positions 2 and 3 in the piperidine ring is a bond and the other dotted line is no bond, Prot' is as defined above and the other residues are as defined above, and

b) removing the protecting Prot' if Prot' is a protecting group to obtain a compound of formula I wherein the dotted line bridging positions 2 and 3 in the piperidine ring is a bond and the other dotted line is no bond, R_5 is hydrogen and the other residues are as defined above; or removing the protecting group from the residue of the protected amino acid if Prot' is the residue of a protected amino acid, to obtain a compound of formula I wherein the dotted line bridging positions 2 and 3 in the piperidine ring is a bond and the other dotted line is no bond, R_5 is the residue of an amino acid and the other residues are as defined above.

In a preferred embodiment a compound of formula II, and, in consequence, e.g. according to step b) to f) of the present invention, a compound of formula I, wherein X is S, one of the dotted line is -OAc, wherein Ac is hydrogen, the other dotted line is no bond and the other residues are as defined above, may be obtained by reaction of a compound of formula



IV

with thiapleuromultilin and Al_2O_3 to obtain a mixture of compounds of formula II, wherein R_6 is a protective group, e.g. BOC and wherein
in one of the compounds of the mixture the hydroxy group is in position 3 and the sulphur group of the thiapleuromutilin is in position 4 of the piperidine ring, and in the other compound of the mixture the hydroxy group is in position 4 and the sulphur group of the thiapleuromutilin is in position 3 of the piperidine ring. That regioisomeric mixture may be

- separated to obtain pure compounds of formula II which pure compounds of formula II may be treated further according to steps b) to f) of the present invention to obtain pure compounds of formula I; or
- the regioisomeric mixture of compounds of formula II may be treated further according to steps b) to f) of the present invention to obtain a mixture of corresponding regioisomers of compounds of formula I which mixture may be separated to obtain pure compounds of formula I.

Separation of regioisomers may be carried out as appropriate, e.g. by chromatography.

If in step A)c) of the present invention the amino acid is used in the (R)-form, e.g.(R)-valine, (R)-histidine, a compound of formula I or II is obtained, wherein the amine group of the

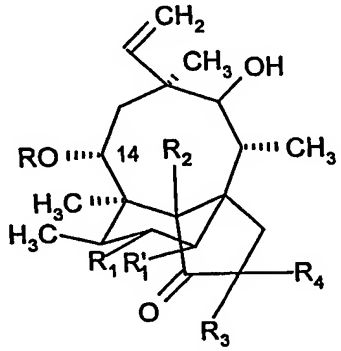
(protected) amino acid group attached to nitrogen atom of the piperidine ring is in the (R)-configuration; and if in step A)c) of the present invention the amino acid is used in the (S)-form, e.g.(S)-valine, (S)-histidine, a compound of formula I or II is obtained, wherein the amine group of the (protected) amino acid group attached to nitrogen atom of the piperidine ring is in the (S)-configuration.

Compounds of formula II are novel and may be useful as intermediates in the production of a compound of formula I, or may be pharmaceutically active.

Protection groups include appropriate protection groups, e.g. such as useful in organic chemistry, e.g. (pleuro)mutilin chemistry, e.g. protection groups as conventional, such as BOC, or -CO-CH_3.

Compounds of formula III, IV, V, VI, VII or VIII are known or may be obtained according to a method as conventional. Any compound described herein may be produced according, e.g. analogously, to a process as conventional, or as described herein.

Replacement of hydrogen atoms in a compound of formula I, e.g. in the form of a salt; by deuterium atoms may be carried out as appropriate, e.g. according to a method as conventional, e.g. or according to a method described herein; e.g. by treatment of a compound of formula I with deuterochloric acid (DCl) in an appropriate solvent (system) and isolation of a compound of formula I, e.g. in the form of a salt, wherein hydrogen atoms, e.g. in the meaning of R_2, R_3 and R_4 are replaced by deuterium atoms. The production of a compound of formula I, wherein R_1 and R'_1 is deuterium may be carried out as appropriate, e.g. according to a method as conventional, e.g. via treatment of a compound of formula



V

wherein the carbon atoms carrying R_1 and R'_1, which both are hydrogen, together form a double bond and wherein R_2, R_3 and R_4 are hydrogen, which is a known compound, with deuterium; to obtain a compound of formula V, wherein R_1 and R'_1 are deuterium and R_2, R_3 and R_4 are hydrogen; and further reacting a compound of formula V, wherein R_1 and R'_1 are deuterium and R_2, R_3 and R_4 are hydrogen as appropriate, e.g. according to a method as

conventional, to obtain a compound of formula II, wherein, R_1 and R'_1 are deuterium and R_2, R_3 and R_4 are hydrogen. R may be a residue which is chemically not affected by deuterium addition, e.g. -CO-CH_2OH.

The compounds of formula I are hereinafter designated as "active compound(s) of the present invention" which exhibit pharmacological activity and are therefore useful as pharmaceuticals. The compound of formula II may be useful intermediates, which may also exhibit pharmacological activity.

For example, the active compounds of the present invention (e.g. and compounds of formula II) show antimicrobial, e.g. antibacterial, activity against gram negative bacterias, such as Escherichia coli; and against gram positive bacteria, such as Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae, Mycoplasms, Chalmydia and obligatory anaerobes, e.g. Bacteroides fragilis; in vitro in the Agar Dilution Test or Microdilution Test according to National Commitee for Clinical Laboratory Standards (NCCLS) 1997, Document M7-A4 Vol.17, No. 2: "Methods for dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically – Fourth Edition, Approved Standard" and e.g. in vivo in systemic infections in mice. The active compounds of the invention show an surprising overall activity spectrum.

In another aspect the present invention provides a compound of formula I, e.g. or of formula II, for use as a pharmaceutical, preferably as an antimicrobial, such as an antibiotic.

In a further aspect the present invention provides a compound of formula I e.g. or of formula II, for use in the preparation of a medicament for the treatment of microbial diseases, for example of diseases caused by bacteria, e.g. selected from Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae, Mycoplasms, Chalmydia e.g. and obligatory anaerobes; e.g. including penicillin or multidrug-resistant strains, e.g. of Streprococcus pneumoniae; e.g. including vancomycin-resistant strains, e.g. of Enterococcus faecium; e.g. and including methicillin-resistant strains, e.g. of Staphylococcus aureus.

In a further aspect the present invention provides a method of treatment of microbial diseases which comprises administering to a subject in need of such treatment an effective amount of a compound of formula I, e.g. or of formula II; e.g. in the form of a pharmaceutical

composition.

For antimicrobial treatment, the appropriate dosage will, of course, vary depending upon, for example, the active compound of the present invention employed, the host, the mode of administration and the nature and severity of the conditions being treated. However, in general, for satisfactory results in larger mammals, for example humans, an indicated daily dosage is in the range from about 0.5 to 3 g, of an active compound of the present invention conveniently administered, for example, in divided doses up to four times a day.
An active compound of the present invention may be administered by any conventional route, for example orally, e.g. in form of tablets or capsules, or parenterally, e.g. in the form of injectable solutions or suspensions, e.g. in analogous manner to erythromycins, such as azithromycin.

The active compounds of the present invention may be administered in the form of a pharmaceutically acceptable salt, e.g. an acid addition salt or metal salt; or in free form; optionally in the form of a solvate. The active compounds of the present invention in the form of a salt exhibit the same order of activity as the active compounds of the present invention in free form.

In another aspect the present invention provides a pharmaceutical composition comprising a compound of formula I, e.g. or of formula II, in free form or in the form of a pharmaceutically acceptable salt; e.g. and/or in the form of a solvate; in association with at least one pharmaceutical, excipient, e.g. carrier or diluent.

Such compositions may be manufactured according to a method as conventional.
Unit dosage form may contain, for example, from about 100 mg to about 1 g.

The active compounds of the present invention are additionally suitable as veterinary agents, e.g. veterinary active compounds, e.g. in the prophylaxis and in the treatment of microbial, e.g. bacterial diseases, in animals, such as fowl, pigs and calves; e.g. and for diluting fluids for artificial insemination and for egg-dipping techniques.

In another aspect the present invention provides a compound of formula I, e.g. or of formula II, for use as a veterinary agent.

In a further aspect the present invention provides a compound of formula I, e.g. or of formula II, for the preparation of a veterinary composition which is useful as a veterinary agent.

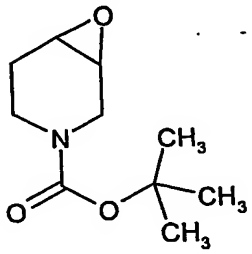
In another aspect the present invention provides a veterinary method for the prophylaxis and in the treatment of microbial, e.g. bacterial diseases which comprises administering to a subject in need of such treatment an effective amount of a compound of formula I, e.g. or of formula II, e.g. in the form of a veterinary composition.

For use of the active compounds of the present invention as a veterinary agent, the dosage will of course vary depending upon the size and age of the animal and the effect desired; for example for prophylactic treatment relatively low doses would be administered over a longer time period, e.g. 1 to 3 weeks. Preferred doses in drinking water are from 0.0125 to 0.05 weight by volume, particularly 0.0125 to 0.025; and in foodstuffs from 20 to 400 g/metric ton, preferably 20 to 200 g/metric ton. It is preferred to administer the active compounds of the present invention as a veterinary agent to hens in drinking water, to pigs in foodstuff and to calves orally or parenterally, e.g. in the form of oral or paraenteral preparations.

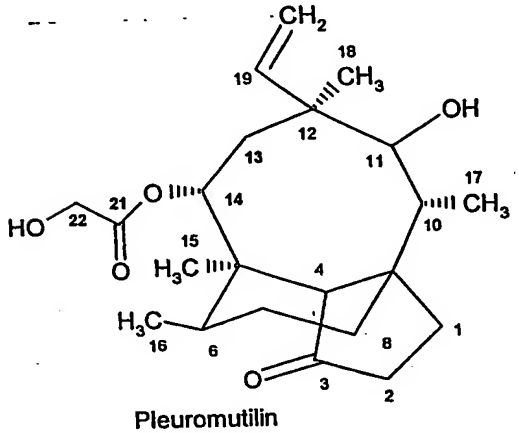
In the following examples all references to temperature are in degrees Centigrade and are uncorrected.

The following abbreviations are used:
BOC = tert.butyloxycarbonyl
DBU: 1,8-diazabicyclo[5.4.0]undec-7-en(1,5-5)
Diast. = diastereoisomer
EDC = N-(3-dimethylaminopropyl)-N-ethylcarbodiimide
EE: ethyl acetate
HOBT = hydroxybenztriazole
RT: room temperature
THF = tetrahydrofurane
TBAF = tetrabutylammoniumfluoride
tert.But-OK = tert.butoxide potassium

N-BOC-3,4-Epoxy-piperidine is a compound of formula

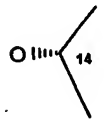


Pleuromutilin is a compound of formula



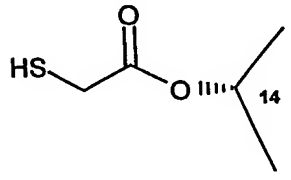
Pleuromutilin

A group of formula

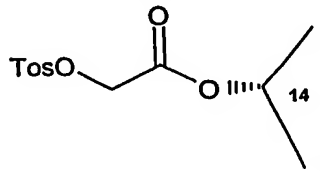


is a group of formula Pleuromutilin, missing the group -CO-CH_2OH.

Thiapleuromutilin is a compound of formula

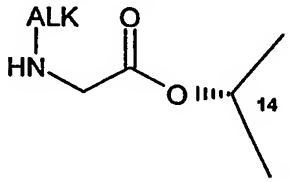


22-O-Tosylpleuromutilin is a compound of formula



wherein Tos is a tosyl group.

HN-alkyl-pleuromutilin is a compound of formula



wherein ALK is (C_{1-4})alkyl, preferably (and in the examples) methyl

Example 1

14-O-[N-BOC-4-Hydroxy-piperidin-3-yl]-sulfanylacetylmutilin and 14-O-[N-BOC-3-hydroxy-piperidin-4-yl]-sulfanylacetylmutilin (compounds of formula II)

40 g of (neutrally) activated Al_2O_3, moistened with THF, are treated with a solution of 1.576 g of thiapleuromutiline in 5 ml of THF and to the mixture obtained 0.398 g of N-BOC-3,4-epoxy-piperidine, dissolved in 3 ml of THF, are added. From the mixture obtained $Al_2O_3$is filtered off, from the filtrate obtained solvent is evaporated off and the evaporation residue comprising a mixture of 14-O-[N-BOC-4-hydroxy-piperidin-4-yl]-sulfanylacetylmutilin and 14-O-[N-Boc-3-hydroxy-piperidin-4-yl]-sulfanylacetylmutilin is subjected to chromatography.

0.156 g of 14-O-[N-BOC-3-Hydroxy-piperidin-4-yl]-sulfanylacetylmutilin (^{1}H-NMR ($CDCl_3$): Diast.: 4.3(b,1H,H_{II}), 4.05(m,1H,H_{VI}), 3.45(m,1H,H_{IV}), 3.28(b,2H,H_{22}), 2.8-2.6(m,2H,H_{II},H_{VI}), 2.55(m,1H,H_{III}), 1.45(s,9H,$(CH_3)_3$)); and

0.05 g of 14-O-[N-BOC-4-Hydroxy-piperidin-4-yl]-sulfanylacetylmutilin (^{1}H-NMR ($CDCl_3$): Diast.: 4.28(m,1H,H_{II}), 4.15-4.0(b,1H,H_{VI}), 3.6-3.32(b,3H,H_{11}), (1.45(s,9H,$(CH_3)_3$))

are obtained.

1.022 g of 14-O-[N-BOC-3-hydroxy-piperidin-4-yl]-sulfanylacetylmutilin are also obtained by reacting 0.466 g of N-BOC-3-hydroxy-4-mercaptopiperidine in 10 ml of THF with 0.224 g of tert.But-OK in 20 ml of THF, adding to the mixture obtained of a solution of 1.064 g of 22-O-tosylpleuromutilin in 5 ml THF, dropwise addition to the mixture obtained of 1 ml of 2-butanone and stirring at RT; and subjecting to chromatographic purification.

Example 2

14-O-[4-Hydroxy-piperidine-3-yl]-sulfanylacetylmutilin (compound of formula I)

1 mmol of 14-O-[N-BOC-4-hydroxy-piperidin-3-yl]-sulfanylacetylmutilin in 5 to 8 ml of CH_2Cl_2 is treated with 1 to 2 ml of etheric HCl, the mixture obtained is stirred at RT and 14-O-[4-hydroxy-piperidine-3-yl]-sulfanylacetylmutilin in the form of a hydrochloride precipitates and is isolated by filtration. (^{1}H-NMR ($CDCl_3$): 3.55-3.15(m,6H,H_{11},H_{22},H_{II},H_{IV},H_{VI}), 2.7-2.55(m,3H,H_{II},H_{III},H_{VI}).

Example 3

14-O-[4-Hydroxy-N-(N-BOC-valyl-piperidin-3-yl]-sulfanylacetylmutilin (compound of formula II)

1.5 mmol of 14-O-[4-hydroxy-piperidine-3-yl]-sulfanylacetylmutilin dissolved in 5 ml of CH_2Cl_2 are treated with 1.5 mmol of HOBT, 1 mmol of (R)-valin and 1.5 mmol of EDC and stirred at RT. From the mixture obtained solvent is evaporated, the evaporation residue obtained is mixed with EE and the mixture obtained is extracted with 0.1N HCl and saturated aqueous $NaHCO_3$ solution. The organic phase obtained is dried and solvent is evaporated. 14-O-[4-Hydroxy-N-(N-BOC-(R)-valyl-piperidin-3-yl-sulfanylacetylmutilin is obtained. (^{1}H-NMR ($CDCl_3$): Rotameres/Diaster.: 5.75(m,1H,NHCO), 4.75, 4.2, 3.95 (3xm,1H,H_{II}), 4.45, 4.35(2xm,1H,NHCO), 3.55(m,1H,H_{IV}), 3.35(m,1H,H_{11}), 3.3(s,2H,H_{22}), 2.55(m,1H,H_{III}), 1.45(b,12H,$(CH_3)_3$, $(CH_3)_{15}$), 0.95, 0.7(2xm,6H,$CH(CH_3)_2$).

Example 4

14-O-[4-Hydroxy-N-(R)-valyl)-piperidine-3-yl]-sulfanylacetylmutilin (compound of formula I)

1 mmol of 14-O-[4-hydroxy-N-(N-BOC-(R)-valyl-piperidin-3-yl-sulfanylacetylmutilin in 5 to 8 ml of CH_2Cl_2 is treated with 1 to 2 ml of etheric HCl, the mixture obtained is stirred at RT and 14-O-[4-Hydroxy-N-(R)-valyl)-piperidine-3-yl]-sulfanylacetylmutilin in the form of the hydrochloride precipitates and is isolated by filtration. (^{1}H-NMR ($CDCl_3$): Diast.: 8.35(b,3H,NH_3^+), 4.5(m,2H,H_{II},NHCHCO), 3.45-3.3(m,3H,H_{11},H_{22}), 2.7, 2.55(2xm,1H,H_{III}), 3.6(m,1H,H_{IV}), 1.1(m,6H,$CH(CH_3)_2$).

Example 5

14-O-[N-(N-BOC-valyl)-1,2,3,6-tetrahydropyridin-3-yl]-sulfanylacetylmutilin (compound of formula II)

a) 3-Mesyloxy-N-(N-BOC-(R)-valyl)-1,2,3,6-tetrahydropyridine

0.894 g of N-(N-BOC-(R)-valyl)-1,2,3,6-tetrahydropyridine-3-ol dissolved in 10 ml of CH_2Cl_2 are treated with 0.844 g of 4-dimethylaminopyridine and 0.31 g of methanesulfonic acid chlorid (mesylchloride) and stirred for ca. 24 hours, the mixture obtained is treated with o.1N HCl and CH_2Cl_2, the organic phase otained is washed with water and aqueous $NaHCO_3$-solution, the solvent is evaporated and the evaporation residue is dried. 3-Mesyloxy-N-(N-BOC-(R)-valyl)-1,2,3,6-tetrahydropyridine is obtained.
(^{1}H-NMR ($CDCl_3$): 6.1-5.85(m,2H,H_{IV},H_V), 4.5(m,1H,NHCHCO), 3.7(s,3H,CH_3SO_2), 1.2-0.9(m,6H,$(CH_3)_2$).

b) 14-O-[N-(N-BOC-(R)-valyl)-1,2,3,6-tetrahydropyridin-3-yl]-sulfanylacetylmutilin

0.235 tert.But-OK dissolved in 5 ml of THF are treated with thiapleuromutilin in 10 ml of THF and to the mixture obtained 0.789 g of 3-mesyloxy-N-(N-BOC-(R)-valyl)-1,2,3,6-tetrahydropyridine in 10 ml of THF are added dropwise. The mixture obtained is heated to 90° and stirred at RT. The mixture obtained is treated with diluted aqueous HCl, the organic phase obtained is washed and solvent is evaporated.
14-O-[N-(N-BOC-(R)-valyl)-1,2,3,6-tetrahydropyridin-3-yl]-sulfanylacetylmutilin is obtained. (^{1}H-NMR ($CDCl_3$): 5.95-5.75(m,2H,H_{IV},H_V), 4.45(m,1H,NHC<u>H</u>CO), 1.45(s,9H,$(CH_3)_3$), 0.9(m,9H,$(CH_3)_{17}$,$(CH_3)_2$).

Example 6

14-O-[N-BOC-1,4,5,6-tetrahydropyridin-4-yl]-sulfanylacetylmutilin

2.72 ml of diisopropylamine in 40 ml of THF are treated with 12 ml n-butyl-lithium (1.6 m solution in hexane) at -40° and the mixture obtained is stirred, warmed to -10° and a solution of 3.44 g of N-BOC-1,2,5,6-tetrahydropyridine in 20 ml of THF is added dropwise. To the mixture obtained a solution of 22-O-tosylpleuromutilin in 10 ml of THF and 1 ml of 2-butanone are added and the mixture obtained is stirred at RT. The mixture obtained comprising a mixture of 14-O-[N-BOC-1,4,5,6-tetrahydropyridin-4(R*)-yl]-sulfanylacetyl-mutilin (COMPOUND A) and 14-O-[N-BOC-1,4,5,6-tetrahydropyridin-4(S*)-yl]-sulfanylacetylmutilin (COMPOUND B) is subjected to chromatography and pure COMPOUND A (^{1}H-NMR ($CDCl_3$): Rotameres: 6.9, 6.7, 4.85, 4.75(4xm,2H,H_{II},H_{III}), 3.8(m,1H, H_{VI}), 3.45(m,1H, H_V), 3.35-3.15(m,3H,H_{11},H_{22}), 2.9(m,1H, H_{IV}), 1.4(b,9H,$(CH_3)_3$)); and pure COMPOUND B (^{1}H-NMR (d_6-DMSO, 350 K): Rotameres: 6.8(d,1H,H_{II},J=8.3Hz), 4.82(dt,1H, H_{III},J=8.3Hz,J=4.9Hz), 4.15(m,1H, H_{VI}), 3.7(m,1H, H_{IV}), 3.55(m,1H, H_{VI}), 3.45, 3.39(2xm,2H, H_V),2xAB-System: v_A=3.32, v_A=3.3,v_B=3.23 v_B=3.21 (2H,H_{22},J=14.8Hz,J=14.9Hz), 1.4 (s,9H,$(CH_3)_3$)); are obtained.

Example 7

14-O-[N-(N-BOC-valyl)-1,4,5,6-tetrahydropyridin-4-yl]-sulfanylacetylmutilin (compound of formula II)

4.53 ml of diisopropylamine in 30 ml of THF are treated with n-butyl-lithium (1.6 m solution in n-hexane) at -40^C. The mixture obtained is stirred, warmed up to -10° and a solution of 5.02 g of 3,4-epithio-N(N-BOC-(R)-valyl)-piperidine in 30 ml of THF is added. The mixture obtained is stirred for ca. 3 hours at -10°, a solution of 22-O-tosylpleuromutilin in 20 ml of THF and 5 ml of 2-butanone are added and the mixture obtained is stirred at RT. The

mixture obtained is subjected to extractive work up and chromatography. 14-O-[N-(N-BOC-(R)-valyl)-1,4,5,6-tetrahydropyridin-4-yl]-sulfanylacetylmutilin is obtained.
^{1}H-NMR ($CDCl_3$): Rotameres/Diast.: 7.25, 6.8, 5.15, 5.05 (4xm,2H,H_{II},H_{III}), 5.3(d,1H,NHCHCO,J=4.6Hz), 4.58(m,1H, H_{IV}), 4.25, 4.05, 3.98(3xd,1H,NHCHCO), 3.65 (m,1H, H_{VI}), 3.5(m,1H, H_V), AB-system: v_A=3.25,v_B=3.15(2H,H_{22},J=15Hz), 1.48 (b,9H,$(CH_3)_3$), 1.0, 0.9(2xd,6H,$CH(CH_3)_2$).

Analogously to a method as described in any one of the preceding examples the following compounds of formula I are obtained:

Example 8: 14-O-[3-hydroxy-piperidin-4-yl]-sulfanylacetylmutilin (^{1}H-NMR ($CDCl_3$): Diast. 3.4(m,1H,H_{III}) 3.35-3.3(m,4H,H_{11},H_{22},H_{VI}), 2.9(m,1H,H_{II}), 2.55(m,1H, H_{IV}).

Examle 9: 14-O-[3-Hydroxy-N-(R)-valyl-piperidin-4-yl]-sulfanylacetylmutilin, e.g. in the form of a hydrochloride (^{1}H-NMR (d_6-DMSO, 350 K): Diast.: 8.05(b,3H,NH_3^+), 4.25–4.1(m, 3H, H_{II}, H_{VI},NHCHCO), 3.[illegible]H,H_{III}), 3.45-3.32(m,3H,H_{11},H_{22}), 2.89(m,1H,H_{IV}), 0.98, 0.92(2xd, 6H,$CH(CH_3)_2$, J=6 Hz).

Example 10: 14-O-[3-Hydroxy-N-(R)-histidinyl-piperidin-4-yl]-sulfanylacetylmutilin, e.g. in the form of a dihydrochloride (^{1}H-NMR (d_6-DMSO, 350 K): Diast.: 8.88, 7.45(2xs,2H, aromat.$H_{imidazol}$), 4.75(m,1H,NHCHCO, AB-System: v_A=3.43, v_B=3.38(2H,H_{22},J=15Hz), 3.48 (d,1H, H_{11},J=6Hz), AB-System: v_A=3.23, v_B=3.15(2H,NHCHCH_2, J=8.3Hz,J=15.6Hz).

Example 11: 14-O-[3-Hydroxy-N-(R)-valyl-piperidin-4-yl]-methylaminoacetylmutilin, e.g. in the form of a dihydrochloride (^{1}H-NMR (d_6-DMSO, 350 K): Diast.: 8.35, 8.15(2xb,4H, CH_3NH^+,NH_3^+), 4.21(b,1H,NHCHCO), 3.35(m,2H,H_{22}), 2.86, 2.83(2xb,3H,CH_3NH^+), 0.94(d,6H,$CH(CH_3)_2$, J=6Hz).

Example 12: 14-O-[4-Hydroxy-N-(R)-valyl-piperidin-3-yl]-methylaminoacetylmutilin, e.g. in the form of a dihydrochloride (^{1}H-NMR (d_6-DMSO): Diast.: 8.3, 8.2(2xb,4H,CH_3NH^+, NH_3^+), 4.1(m,1H,NHCHCO), 3.45(b,2H,H_{22}), 2.95, 2.9(2xs,3H,CH_3NH^+), 0.95(m,6H,$CH(CH_3)_2$).

Example 13: 14-O-[N-valyl)-1,2,3,6-tetrahydropyridin-3-yl]-sulfanylacetylmutilin

a) 14-O-[N-(R)-valyl-1,2,3,6-tetrahydropyridin-3(R*)-yl]-sulfanylacetylmutilin
(^{1}H-NMR ($CDCl_3$): Rotameres: 5.95-5.75(m,3H,H_{14},H_{IV},H_V), 2xAB-system: v_A=4.22, v_A=4.09, v_B=3.9, v_B=4.0(2H,H_{VI},J=19.2Hz), AB-system: v_A=4.2, v_B=3.77(2H,H_{II},J=17.7Hz), 3.68-3.6(m,1H,H_{III}), 3.52(m,1H,NHCHCO), 3.2(m,2H,H_{22}).
H_{22},$J_{22,sH}$=8.2Hz,J_{AB}=15.1Hz,J_{AX}= 8.2Hz),
b) 14-O-[N-(R)-valyl-1,2,3,6-tetrahydropyridin-3(S*)-yl]-sulfanylacetylmutilin
(^{1}H-NMR ($CDCl_3$): Rotameres: 5.98-5.78(m,2H,H_{IV},H_V), 5.78(d,1H,H_{14},J=8.4Hz), 3xAB-system: v_A=4.7,v_A=4.61,v_A=4.5,v_B=3.8,v_B=3.7,v_B=3.42 (2H,H_{VI},J_1=19.5Hz,J_2=18.9Hz, J_3=14.4Hz), 3xAB-system: v_A=4.35,v_A=4.1,v_A=3.88,v_B=3.98,v_B=3.7,v_B=3.72,v_B=3.46 (2H,H_{II},J_1=13.7Hz,J_2=13.7Hz, J_3=13.9Hz), 3.65(m,1H,H_{III}), 3.58(m,1H,NHCHCO).

Example 14: 4-O-[4-Acetoxy-N(R)-valyl)-piperidin-3-yl]-sulfanylacetylmutilin
(^{1}H-NMR (d_6-DMSO): Diast.: 8.1(b,3H,NH_3^+), 4.52(m,1HH_{IV}), 4.32, 4.28(2xm,1H,NHCHO), 3.5-3.35(m,4H,H_{11},H_{22},H_{VI}), 2.93, 2.88(2xm,1H,H_{II}), 2.03, 2.02, 2.00(3xs,3H,$OCOCH_3$), 0.98, 0.88(2xm,6H,$CH(CH_3)_2$).

Analogously to a method as described in any one of the preceding examples the following compounds of formula II are obtained:

Example 15: 14-O-[3-hydroxy-N-(N-BOC-(R)-valyl)-piperidin-4-yl]-sulfanylacetylmutilin, e.g. in the form of a hydrochloride (^{1}H-NMR ($CDCl_3$): Rotameres/Diast.: 6.8, 6.68(2m,1H, NHCHCO), 5.32(m,1H,OH), 4.2(m,1H,NHCHCO), 3.85(m,1H,H_{VI}), 3.5-3.3(m,3H,H_{11},H_{22}), 3.15(m,1H,H_{III}), 2.8(m,1H,H_{IV}), 1.35(s,12H,$(CH_3)_3$,$(CH_3)_{15}$), 0.8(m,9H,$CH(CH_3)_2$),$(CH_3)_{17}$).

Example 16: 14-O-[3-hydroxy-N-(N-BOC-(R)-histidinyl-piperidin-4-yl]-sulfanylacetyl-mutilin (^{1}H-NMR (d_6-DMSO, 350 K): Diast.: 8.21, 8.02(2xs,2H, aromat.$H_{imidazol}$), 7.18(d,1H,NHCHCO,J=3.1 Hz), 6.55 (b,1H,OH), 4.65(m,1H,H_{VI}), H4,15 (m, 1H,NHCHCO), 3.5-3.1(m, 5H,$NHCHCH_2$,H_{11},H_{22}), 2.8(m,1H,H_{IV}), 1.55, 1.35(2xs,18H,2x$(CH_3)_3$).

Example 17: 14-O-[4-hydroxy-N-BOC-piperidin-3-yl]-methylaminoacetylmutilin
^{1}H-NMR ($CDCl_3$): Diast.: 4.2-4.0(b,2H,H_{II},H_{VI}), 3.5(m,1H,H_{IV}), 3.4-3.2(m,3H,H_{11},H_{22}), 2.65, 2.5(2xm,2H,H_{II},H_{VI}), 2.42(s,3H,NCH_3), 1.45(s,12H,$(CH_3)_3$(CH_3)$_{15}$).

Example 18: 14-O-[3-hydroxy-N-BOC-piperidin-4-yl]-methylaminoacetylmutilin

^{1}H-NMR ($CDCl_3$): Diast.: 4.4, 4.2(2xm,2H,H_{II},H_{VI}), 3.4-3.12(m,4H,H_{11},H_{22},H_{III}), 2.58, 2.49(2xm,2H,H_{II},H_{VI}), 2.38(s,3H,NCH_3), 1.45(b,12H,$(CH_3)_3(CH_3)_{15}$).

Example 19: 14-O-[4-Acetoxy-N-(N-BOC-(R)-valyl)-piperidin-3-yl]-sulfanylacetylmutilin

^{1}H-NMR (d_6-DMSO): Diast.: 8.1(b,3H,NH_3^+), 4.52(m,1H,H_{IV}), 4.32, 4.28(2xm,1H,NHCHCO), 3.5-3.35(m,4H,H_{11},H_{22},H_{VI}), 2.93,2.88(2xm,1H,H_{III}), 2.03, 2.02, 2.01(3s,3H,$OCOCH_3$). 0.98, 0.88(2xm,6H,$CH(CH_3)_2$).

Example 20: 14-O-[3-Acetoxy-N-(N-BOC-(R)-valyl)-piperidin-4-yl]-sulfanylacetylmutilin

^{1}H-NMR (d_6-DMSO): Diast.: 8.05(b,3H,NH_3^+),4.62(m,1H,NHCHCO), 4.52(m,1H,H_{III}), 4.25, 4.18(2xm,1H,H_{VI}), AB-system: v_A=3.95,v_B=3.65(2H,H_{II},J=2.8Hz,J=12.6Hz),3.4(m,3H,H_{11},H_{22}), 3.12(m,1H,H_{IV}), 0.98, 0.88(2xm,6H,$CH(CH_3)_2$).

Example 21: 14-O-[4-hydroxy-N-(N-BOC)-(R)-valyl-piperidin-3-yl]-methylamino-acetylmutilin (^{1}H-NMR ($CDCl_3$): Diast.: 4.2-4.0(b,2H,H_{II},H_{VI}), 3.5(m,1H,H_{IV}), 3.4-3.2 (m,3H,H_{11},H_{22}), 2.65, 2.5(2xm,2H,H_{II},H_{VI}), 2.42(s,3H,NCH_3), 1.45(s,12H,$(CH_3)_3(CH_3)_{15}$).

Example 22: 14-O-[3-hydroxy-N-(N-BOC)-(R)-valyl-piperidin-4-yl]-methylamino-acetylmutilin (^{1}H-NMR ($CDCl_3$): Diast.: 4.4, 4.2(2xm,2H,H_{II},H_{VI}), 3.4-3.12(m,4H,H_{11},H_{22}, H_{III}), 2.58-2.49(2xm,2H,H_{II},H_{VI}), 2.38(s,3H,NCH_3), 1.45(b,12H,$(CH_3)_3(CH_3)_{15}$).

Production of starting material

Example A - Thiapleuromutilin

a) Thiapleuromutilin in the form of the isothiuronium salt

A mixture of 106.4 g of 22-O-tosylpleuromutilin, 15.2 g of thiourea and 250 ml of aceton is refluxed for ca. 1.5 hours, cooled and from the mixture obtained solvent is evaporated and the evaporation residue is dried in vacuo. Thiapleuromutilin in the form of an isothiuronium salt is obtained.

^{1}H-NMR ($CDCl_3$): 9.82, 8.42(2xb,2H,NH_2), 7.78, 7.2(2xd,4H,arom.H_{Tosyl};J=15.8Hz).

a) Thiapleuromutilin

24.4 g of thiapleuromutilin in the form of an isothiuronium salt, dissolved in 40 ml absolute EtOH, is diluted with 70 ml of water and warmed to 90°. The mixture obtained is treated with 7.6 g of sodium disulfite in 35 ml of water and to the mixture obtained 200 ml of CH_2Cl_2 are added. The mixture obtained is heated to 90° for ca. 1.5 hours and cooled. Two phases are

formed and are separated, the organic phase obtained is washed, dried, solvent is evaporated and the evaporation residue is filtered through silicagel. 8.16 g of thiapleuromutilin are obtained.

^{1}H-NMR ($CDCl_3$): 6.48(dd,1H,H_{19},$J_{19,20cis}$=11Hz,$J_{19,20trans}$=16.5Hz), 5.75(d,1H,H_{14},$J_{13,14}$= 8.5Hz), 5.38(dd,1H,H_{20},$J_{20,20}$= 1.5Hz), 5.2(dd,1H,$H_{20trans}$), 3.38(dd,1H,H_{11},$J_{11,OH}$=10.4Hz, $J_{11,10}$=6.6Hz), ABX-System: ν_A=3.21, ν_B=3.18,ν_X=1.9 (H_{22},$J_{22,sH}$=8.2Hz,J_{AB}=15.1Hz,J_{AX}= 8.2Hz), 2.35(quint.1H,H_{10},$J_{10,17}$=8.2Hz), 2.28, 2.2(2H, $H_{H2\alpha,2\beta}$,$J_{2\alpha,2\beta}$=15.5Hz,$J_{2\alpha,1\alpha}$=$J_{2\alpha,1\beta}$= 5.5Hz), 2.19(dd,1H,H_{13},$J_{13,13}$=16Hz,$J_{13,14}$=8.5Hz), 2.12(b,1H,H_4), 1.9(t,1H,SH,$J_{22,sH}$=8.2Hz), 1.79, 1.76(2xq,1H,$H_{8equ.}$,$J_{7,8equ}$=3.01Hz,$J_{8,8}$=14.5Hz), 1.67(m,2H,H_1,H_6), 1.57, 1.53(2xm,1H, H_{7ax}), 1.45(s,3H,$(CH_3)_{15}$), 1.39, 1.36(2xq,1H,H_{7q},$J_{7,7}$=7.23Hz), 1.33(d,1H,$H_{13'}$), 1.18(s,3H, $(CH_3)_{18}$), 1.12(dd,1H,H_{8ax},$J_{7,8ax}$=1.14Hz), 0.89(d,3H,$(CH_3)_{17}$,$J_{10,17}$=6.54Hz), 0.74(d,3H, $(CH_3)_{16}$,$J_{6,16}$=6.5Hz). ^{1}H-NMR (d_6-DMSO): 2.85(s,1H,SH).

Example B - N-BOC-3,4-Epoxy-piperidine

a) N-BOC-1,2,5,6-tetrahydropyridine

To 1.66 g of 1,2,5,6-tetrahydropyridine in 25 ml of CH_2Cl_2, [illegible] of N-methylmorpholine are added, the mixture obtained is treated with a solution of 4.36 g $(BOC)_2O$ in 30 ml of CH_2Cl_2 and the mixture obtained is stirred for ca. 36 hours at RT. 3.4 g of N-BOC-1,2,5,6-tetrahydropyridine are obtained. ^{1}H-NMR ($CDCl_3$): 5.82(m,1H,H_{IV}), 5.64(m,1H,H_{III}), 3.86(b,2H, H_{II}), 3.47(t,2H,H_{VI}), 2.12(b,1H,H_V), 1.46(m,9H,$(CH_3)_3$).

b) N-BOC-3,4-Epoxy-piperidine

To a solution of 3.29 g of N-BOC-1,2,5,6-tetrahydropyridine in 25 ml of CH_2Cl_2, a suspension of 6.2 g of chloroperbenzoic acid in 50 ml of CH_2Cl_2 are added and the mixture obtained is stirred for ca. 12 hours at RT. The mixture obtained is extracted with saturated aqueous $NaHCO_3$-solution and 0.5 m aqueous $Na_2S_8O_3$-solution and the organic phase obtained is washed, dried and the solvent is evaporated. 3.41 g of N-BOC-3,4-epoxy-piperidine are obtained. ^{1}H-NMR ($CDCl_3$): 3.9, 3.65, 3.45, 3.1(4xm,4H,H_{II},H_{VI}), 3.28, 3.2 (2xm,2H,H_{III},H_{IV}), 2.05, 1.9(2xm,2H,H_V), 1.45(s,9H,$(CH_3)_3$).

Example C - N-(N-BOC-(R)-valyl)-1,2,3,6-tetrahydropyridine-3-ol

a) N-(N-BOC-valyl-1,2,5,6-tetrahydropyridine

1.245 g of tetrahydropyridine in 50 ml of CH_2Cl_2 are treated with 1.5 mmol per mmol of tetrahydropyridine of HOBT, 2.17 g of N-BOC-(R)-valin and 1.5 mmol per mmol of tetrahydropyridine of EDC and the mixture obtained stirred at RT. From the mixture obtained

solvent is evaporated, the evaporation residue obtained is mixed with EE and the mixture obtained is extracted with 0.1N HCl and saturated aqueous $NaHCO_3$ solution. The organic phase obtained is dried and solvent is evaporated. N-(N-BOC-(R)-valyl-1,2,5,6-tetrahydropyridine is obtained.

b) 3,4-Epoxy-N-(N-BOC-valyl-1,2,5,6-tetrahydropyridine

To a solution of 2.82 g og N-(N-BOC-(R)-valyl-1,2,5,6-tetrahydropyridine in 75 ml of CH_2Cl_2, 3.44 g of m-chloroperbenzoic acid in 50 ml of CH_2Cl_2 are slowly added and the mixture obtained is stirred overnight. The mixture obtained is extracted with aqueous NaHCO3-solution and with 0.5 m aqueous Na2S2O3-solution, the phases obtained are separated and from the organic phase solvent is evaporated in vacuo. 2.49 g of 3,4-Epoxy-N-(N-BOC-(R)-valyl-1,2,5,6-tetrahydropyridine are obtained.

^{1}H-NMR ($CDCl_3$): Rotameres: 5.3(m,1H,NHCHCO), 4.4(m,1H,NHCHCO), 4.3, 4.1, 4.0 (3dd,1H,H_{III},J=15.6Hz), 3.88, 3.78, 3.65(3xd,1H,H_{IV},J=15.6Hz), 3.6, 3.45, 3.3(3xm,4H,H_{II}, H_{VI}), 1.45(b,9H(CH_3)$_3$), 1.0-0.85(m,6H,CH(CH_3)$_2$).

c) Bromo-N-(N-BOC-valyl)-piperidin-3-ol

0.5 g of Ph_3PBr_2 in 10 ml of CH_2Cl_2 are treated with 0.289 g of 3,4-epoxy-N-(N-BOC-(R)-valyl-1,2,5,6-tetrahydropyridine in 10 ml of CH_2Cl_2. The mixture obtained is poured onto a mixture of ice/NaHCO3, the organic phase is separated, washed, dried and solvent is evaporated. A mixture of 4(R*)-bromo-N-(N-BOC-(R)-valyl)-piperidin-3(R*)-ol (COMPOUND A) and 4(S*)-bromo-N-(N-BOC-(R)-valyl)-piperidin-3(S*)-ol (COMPOUND B) is obtained and separated by chromatography.

COMPOUND A: ^{1}H-NMR ($CDCl_3$): Rotameres: 5.2(m,1H,NHCHCO), 4.3(t,1H,NHCHCO,J=6.5Hz), 4.25(m,1H,H_{IV}), 3.88(m,1H,H_{III}), 2.4, 1.85(2xm,2H,H_V), 1.43(b,9H(CH_3)$_3$), 0.98, 0.92(2xd,6H,CH(CH_3)$_2$,J=7Hz).

COMPOUND B: ^{1}H-NMR ($CDCl_3$): Rotameres: 5.25(d,1H,NHCHCO,J=6.7Hz), 4.45(m,1H,NHCHCO), 4.15(m,1H,H_{IV}), 3.75(m,1H,H_{III}), 2.55, 2.3(2xm,2H,H_V), 1.9(m,1H,CH(CH_3)$_2$), 1.42 (b,9H(CH_3)$_3$), 0.9(m,6H,CH(CH_3)$_2$).

d) 3-Acetoxy-4-bromo-N-(N-BOC-valyl)-piperidine

0.57 g of bromo-N-(N-BOC-valyl)-piperidin-3-ol, dissolved in pyridine, is treated with 0.4 ml of acetic acid anhydride, the mixture obtained is stirred and a mixture of 3(R*)-acetoxy-4(R*)-bromo-N-(N-BOC-(R)-valyl)-piperidine (COMPOUND A) and 3(S*)-acetoxy-4(S*)-bromo-N-(N-BOC-(R)-valyl)-piperidine (COMPOUND B) is obtained and is separated by chromatography.

COMPOUND A: ^{1}H-NMR (d_6-DMSO, 350 K): 6.4(b,1H,NHCHCO), 4.73(dt,1H,NHCHCO, J=3.9Hz,J=7.7Hz), 4.38(dt,1H,H_{III},J=4.4Hz,J=8.8Hz), 4.18(m,1H,NHCHCO), 4.05, 3.8, 3.35(3m,4H,H_{II},H_{VI}), 2.3(s,3H,$OCOCH_3$), 1.38(s,9H(CH_3)$_3$), 0.85(d,6H,CH(CH_3)$_2$,J=7Hz).

COMPOUND B: ^{1}H-NMR (d_6-DMSO, 350 K): 6.5(b,1H,NHCHCO), 4.72(dt,1H,H_{IV},J=4.0Hz, J=7.7Hz), 4.38(dt,1H,H_{III},J=4.4Hz,J=8.6Hz), 4.2(m,1H,NHCHCO),4.11, 3.78, 3.3(3m,4H,H_{II},H_{VI}), 2.3(s,3H,$OCOCH_3$), 1.37(s,9H,(CH_3)$_3$), 0.85(d,6H,CH(CH_3)$_2$,J=7Hz).

e) 3-Acetoxy-N-(N-BOC-(R)-valyl)-1,2,3,6-tetrahydropyridine

1.684 g of 3-acetoxy-4-bromo-N-(N-BOC-valyl)-piperidine dissolved in 4 ml of toluene are treated with 4 ml of DBU in a sealed tube and heated to 90°. The mixture obtained is treated with EE, extracted with aqueous HCl, washed and from the organic phase obtained solvent is evaporated. 3-Acetoxy-N-(N-BOC-(R)-valyl)-1,2,3,6-tetrahydropyridine is obtained.

^{1}H-NMR ($CDCl_3$): Rotameres/Diast: 5.95, 5.85, 5.25, 5.15(4xm,2H,H_{IV},H_V), 4.51, 4.4(2xdd, 1H,NHCHCO,J=5.2Hz,J=9Hz), 4.45, 4.15(2xd,1H,H_{VI},J=15.2Hz), 3.4, 3.2(2xdd,1H,H_{VI}, J=3.5Hz),2.02, 2.0, 1.95(3xs,3H,$OCOCH_3$), 1.35(s,9H,(CH_3)$_3$), 0.85(m,6H,CH(CH_3)$_2$).

f) N-(N-BOC-(R)-valyl)-1,2,3,6-tetrahydropyridine-3-ol

0.25 [illegible]-acetoxy-N-(N-BOC-(R)-valyl)-1,2,3,6-tetrahydropyridine, dissolved in 5 ml of EtOH are treated with 2N ethanolic NaOH under ice-cooling. To the mixture obtained acetic acid is added in order to neutralize the reaction mixture and solvent is evaporated. The evaporation resdue obtained is mixed with CHCl3, the mixture obtained is washed with NaCl-solution, the organic phase is dried and solvent is evaporated. 0.209 g of N-(N-BOC-(R)-valyl)-1,2,3,6-tetrahydropyridine-3-ol are obtained.

^{1}H-NMR ($CDCl_3$): 5.9(m,2H,H_{IV}, H_V), 4.51, 4.45(2xdd,1H,NHCHCO,J=5.2Hz,J=9.0Hz), 1.4 (b,9H,(CH_3)$_3$), 0.9(m,6H,CH(CH_3)$_2$).

Example D - Methylaminoacetylmutilin

13.33 g of 22-O-tosylpleuromutilin in 350 ml of EtOH are treated with 5 ml CH_3NH_2 (33% solution in EtOH), the mixture obtained is refluxed for ca. 30 hours and from the mixture obtained solvent is evaporated. The evaporation residue is treated with EE and the mixture obtained is extracted with 0.1N HCl. The aqueous phase obtained is treated with $NaHCO_3$ and extracted with EE. The organic phase obtained is dried and solvent is evaporated. 3.83 g of methylaminoacetylmutilin are obtained.

^{1}H-NMR ($CDCl_3$): AB-system: ν_A=3.32,ν_B=3.22(2H,H_{22},$J_{22,NCH3}$=15Hz), 2.42(s,3H,CH_3NH).

Example E

N-BOC-1,2,5,6-tetrahydropyridine

1.66 g of 1,2,5,6-tetrahydropyridine in 25 ml of CH_2Cl_2 are treated with 2.02 g of N-methylmorpholine. To the mixture obtained 4.36 g of $(BOC)_2O$ in 30 ml of CH_2Cl_2 are added and the mixture obtained is left for reaction for ca. 36 hours. The mixture obtained is subjected to aqueous extraction, the organic phase is dried and evaporated. 3.4 g of N-BOC-1,2,5,6-tetrahydropyridine are obtained.

^{1}H-NMR ($CDCl_3$): 5.82(m,1H,H_{IV}), 5.64(m,1H,H_{III}), 3.86(b,2H,H_{II}), 3.47(t,2H,H_{VI}), 2.12(b,1H,H_V), 1.46(m,9H,$(CH_3)_3$).

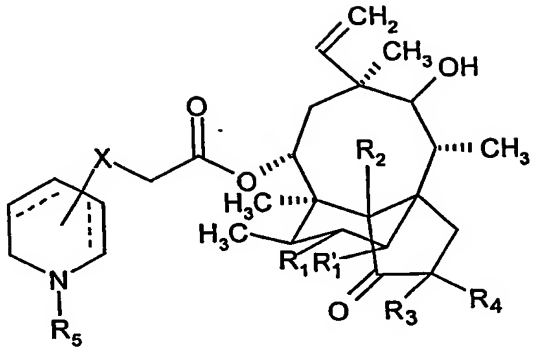
Example F

3,4-Epithio-N(N-BOC-valyl)-piperidine

To a mixture of 5.96 g of 3,4-epoxy-N-(N-BOC-valyl-1,2,5,6-tetrahydropyridine in 10 ml of absolute EtOH 2.91 g of KSCN in 3 ml of water are added and the mixture obtained is stirred for 72 hours at RT. The mixture obtained is subjected to aqueous extraction and the solvent of the organic phase obtained is evaporated and the evaporation residue is subjeted to chromatography. 6.21 g of 3,4-Epithio-N(N-BOC-(R)-valyl)-piperidine are obtained. Melting point: 69.71°

Patent claims

1. A compound of formula



I

wherein

R_1 and R_1' are hydrogen or deuterium,

R_2, R_3 and R_4 are hydrogen or deuterium,

R_5 is hydrogen or a residue of an amino acid,

X is S or N-ALK,

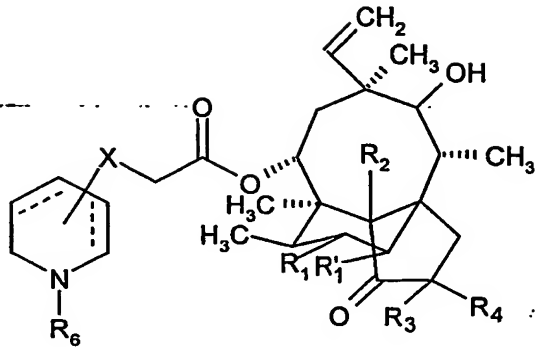
one of the dotted lines is a bond and the other is no bond; [illegible] of the dotted lines is a group -OAc attached to the piperidine ring in position 2, 3, 4, 5 or 6, and the other dotted line is no bond,

ALK is (C_{1-4})alkyl, e.g. methyl, and

Ac is hydrogen or (C_{2-12})acyl, e.g. a group -CO-CH_3.

2. A compound of formula I which is selected from the group consisting of
14-O-[4-hydroxy-piperidin-3-yl-sulfanylacetylmutilin,
14-O-[3-hydroxy-piperidin-4-yl-sulfanylacetylmutilin,
14-O-[4-hydroxy-N-valyl-piperidin-3-yl]-sulfanylacetylmutilin,
14-O-[3-hydroxy-N-valyl-piperidin-4-yl]-sulfanylacetylmutilin,
14-O-[3-hydroxy-N-histidinyl-piperidin-4-yl]-sulfanylacetylmutilin,
14-O-[3-hydroxy-N-valyl-piperidin-4-yl]-methylaminoacetylmutilin,
14-O-[4-hydroxy-N-valyl-piperidin-3-yl]-methylaminoacetylmutilin,
14-O-[N-valyl)-1,2,3,6-tetrahydropyridin-3-yl]-sulfanylacetylmutilin, and
14-O-[N-valyl)-1,4,5,6-tetrahydropyridin-4-yl]-sulfanylacetylmutilin.

3. A compound of formula



II

wherein

R_1 and R_1' are hydrogen or deuterium,

R_2, R_3 and R_4 are hydrogen or deuterium,

R_6 is a protective group, or the residue of a protected amino acid,

X is S or N-ALK,

one of the dotted lines is a bond and the other is no bond; or one of the dotted lines is a group -OAc attached to the piperidine ring in position 2, 3, 4, 5 or 6, and the other dotted line is no bond,

ALK is (C_{1-4})alkyl, and

Ac is (C_{2-12})acyl.

4. A compound of formula II selected from the group consisting of

14-O-[N-BOC-4-hydroxy-piperidin-3-yl]-sulfanylacetylmutilin,

14-O-[N-BOC-3-hydroxy-piperidin-4-yl]-sulfanylacetylmutilin,

14-O-[4-hydroxy-N-BOC-piperidin-3-yl]-methylaminoacetylmutilin,

14-O-[3-hydroxy-N-BOC-piperidin-4-yl]-methylaminoacetylmutilin,

14-O-[N-BOC-1,4,5,6-tetrahydropyridin-4-yl]-sulfanylacetylmutilin,

14-O-[4-hydroxy-N-(N-BOC-valyl)-piperidin-3-yl]-sulfanylacetylmutilin,

14-O-[3-hydroxy-N-(N-BOC-valyl)-piperidin-4-yl]-sulfanylacetylmutilin,

14-O-[4-acetoxy-N-(N-BOC-valyl)-piperidin-3-yl]-sulfanylacetylmutilin,

14-O-[3-acetoxy-N-(N-BOC-valyl)-piperidin-4-yl]-sulfanylacetylmutilin,

14-O-[3-hydroxy-N-(N-BOC-histidinyl)-piperidin-4-yl]-sulfanylacetylmutilin,

14-O-[3-hydroxy-N-(N-BOC)-valyl-piperidin-4-yl]-methylaminoacetylmutilin,

14-O-[4-hydroxy-N-(N-BOC)-valyl-piperidin-3-yl]-methylaminoacetylmutilin,

14-O-[N-(N-BOC-valyl)-1,4,5,6-tetrahydropyridin-4-yl]-sulfanylacetylmutilin,

14-O-[N-(N-BOC-valyl)-1,2,3,6-tetrahydropyridin-3-yl]-sulfanylacetylmutilin.

5. A compound according to any one of claims 1 to 4 in the form of a salt, or in the form of a salt and in the form of a solvate, or in the form of a solvate.

6. A compound according to any one of claims 1 to 5 for use as a pharmaceutical.

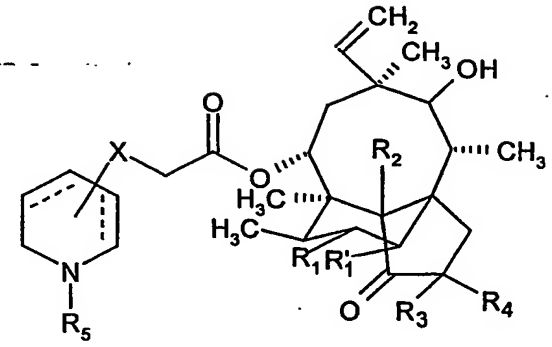
7. A method of treatment of microbial diseases comprising administering to a subject in need of such treatment an effective amount of a compound of any one of claims 1 to 5.

8. A pharmaceutical composition comprising a compound of any one of claims 1 to 5 in association with at least one pharmaceutical excipient.

SC/24-Jul-02

Abstract

A compound of formula



I

wherein the residues ave various meanings and its use as a pharmaceutically active compound.